

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1-11 (cancelled).

12 (currently amended). An isolated polypeptide which

~~(a) comprises SEQ ID NO:2;~~

(a) ~~(b)~~ consists of residues 234-1791 of SEQ ID NO:2; or

~~(c) differs from the polypeptide of (b) solely by deletion of 1-10 residues from the amino terminal of (b) and/or deletion of 1-10 residues from the carboxy terminal of (b);~~

~~(d) differs from (b) solely by addition of 1-10 residues to the amino terminal or carboxy terminal of (b); or~~

(b) ~~(e)~~ is at least ~~97%~~ 99% identical to a polypeptide of (a), ~~(b)~~, ~~(c)~~, ~~or (d)~~ but differs therefrom by one or more insertions and/or deletions and/or substitutions,

wherein said polypeptide

i) has a proteolytic activity ~~specific at least for~~ against Insulin Like Growth Factor Binding Protein 5 (IGFBP-5); and/or

ii) is recognised by an antibody, or a binding fragment thereof, which recognizes a mature pregnancy associated plasma protein A-2 (mature PAPP-A2) ~~polypeptide having~~ consisting of the amino acid sequence as shown as in amino acids 234-1791 of SEQ ID NO:2; and/or

~~iii) competes with a polypeptide having the amino acid~~

~~sequence as shown in SEQ ID NO:2 for binding to a cell surface receptor with an affinity for said polypeptide.~~

13-17 (cancelled).

18. (previously presented). The polypeptide of claim 12, wherein said polypeptide is a recombinant polypeptide.

19. (previously presented). The polypeptide of claim 12, wherein the polypeptide is free of human proteins, or other proteins naturally associated with said polypeptide.

20-29 (cancelled).

30 (withdrawn-currently amended). A method for detecting pregnancy associated plasma protein A-2 (PAPP-A2), or measuring the level of PAPP-A2, in a biological sample obtained from an individual, said method comprising the steps of

- i) obtaining a biological sample from said individual,
- ii) detecting PAPP-A2 in said sample by detecting a polypeptide according to claim 12.

31 (withdrawn). Method of claim 30, said method comprising the further step of comparing the PAPP-A2 or the level of PAPP-A2 detected in step ii) with a predetermined value selected from the group consisting of

- i) a predetermined amount and/or concentration of PAPP-A2; and/or
- ii) a predetermined amount and/or concentration of PAPP-A2 mRNA; and/or
- iii) a predetermined PAPP-A2 specific protease activity.

32 (withdrawn). Method of claim 31, wherein said predetermined value is indicative of a normal physiological condition of said individual.

33 (withdrawn). The method of claim 30, wherein said biological sample is selected from the group consisting of blood, urine, pleural fluid, oral washings, tissue biopsies, and

follicular fluid.

34 (withdrawn). The method of claim 30, wherein said level of PAPP-A2 is measured as PAPP-A2 specific protease activity.

35 (withdrawn). The method of claim 30, wherein said level of PAPP-A2 is measured as amount of PAPP-A2 protein.

36 (withdrawn). The method of claim 30, wherein said level of PAPP-A2 is measured as amount of PAPP-A2 messenger RNA.

37 (withdrawn). The method of claim 35, wherein said amount of PAPP-A2 protein is measured by immunochemical analysis.

38 (withdrawn). The method of claim 37, wherein said amount of PAPP-A2 protein is detected by at least one monoclonal antibody.

39 (withdrawn-currently amended). The method of claim 30, wherein said PAPP-A2 protein is detected in a complex comprising at least one additional ~~component, preferably a~~ polypeptide.

40 (withdrawn). The method of claim 30, wherein said PAPP-A2 is detected as a PAPP-A2 monomer.

41 (withdrawn). The method of claim 30, wherein said PAPP-A2 is detected as a PAPP-A2 dimer.

42 (withdrawn). A method of diagnosing a clinical condition in an individual, said method comprising the steps of

i) performing the method of claim 30, and

ii) diagnosing the clinical condition.

43 (withdrawn). Method of claim 42, wherein said clinical condition is a fetal abnormality.

44 (withdrawn). The method of claim 43, wherein said fetal abnormality is selected from the group consisting of Trisomy 21, Trisomy 18, Trisomy 13, and Open Spina Bifida.

45 (withdrawn). The method according to claim 43, wherein said fetal abnormality is ectopic pregnancy, open spina bifida, neural tube defects, ventral wall defects, Edwards Syndrome, Patau's Syndrome, Turner Syndrome, Monosomy X or Klinefelter's Syndrome.

46 (withdrawn). The method of claim 43, wherein said clinical condition is an altered growth state selected from the group consisting of a growth promoting state and a growth inhibiting state.

47 (withdrawn). The method of claim 46, wherein said clinical condition is selected from the group consisting of restenosis, atherosclerosis, wound healing, fibrosis, myocardial infarction, osteoporoses, rheumatoroid arthritis, multiple myeloma, or cancer.

48 (cancelled).

49 (withdrawn-currently amended). A method for identifying an agent inhibiting the protease activity of pregnancy associated plasma protein A-2 (PAPP-A2), said method comprising the steps of

- i) incubating a) the polypeptide according to claim 12 and b) a predetermined substrate for said polypeptide, and c) a putative inhibitory agent, and
- ii) determining if proteolysis of said substrate is inhibited.

50 (withdrawn). The method of claim 49, wherein said substrate comprises a polypeptide.

51 (withdrawn). The method of claim 50, wherein said substrate comprises an internally quenched fluorescent peptide.

52 (withdrawn-currently amended). The method of claim 50, wherein said substrate comprises ~~or essentially consists of~~ IGFBP-5, or a fragment thereof.

53-54 (cancelled).

55 (withdrawn-currently amended). A method for identifying an agent enhancing the protease activity of pregnancy associated plasma protein A-2 (PAPP-A2), said method comprising the steps of

- i) incubating a) the polypeptide according to claim 12 and b) a predetermined substrate for said polypeptide, and c) a putative enhancer agent, and
- ii) determining if proteolysis of said substrate is

enhanced.

56 (withdrawn). The method of claim 55, wherein said substrate comprises a polypeptide.

57 (withdrawn). The method of claim 55, wherein said substrate comprises an internally quenched fluorescent peptide.

58 (withdrawn-currently amended). The method of claim 55, wherein said substrate comprises ~~or essentially consists of~~ IGFBP-5, or a fragment thereof.

59-61 (cancelled).

62 (withdrawn-currently amended). A method for purification of pregnancy associated plasma protein A-2 (PAPP-A2) or complexes of PAPP-A2 with other proteins, said method comprising the steps of

- i) providing a polyclonal or monoclonal antibody with specific binding affinity for a polypeptide according to claim 12,
- ii) purifying PAPP-A2 by means of affinity chromatography.

63-74 (cancelled).

75 (currently amended). The polypeptide of claim 12 wherein said polypeptide is the polypeptide according to ~~(a)~~ (b).

76-82 (cancelled).

83 (currently amended). The polypeptide of claim ~~12~~ 75 wherein said polypeptide comprises an amino acid sequence which differs from said sequence (a) ~~(b)~~ solely by one or more conservative substitutions.

84-86 (cancelled).

87 (previously presented). The polypeptide of claim 12 wherein said polypeptide consists of amino acids 234 to 1791 of SEQ ID NO:2.

88-89 (cancelled).

90 (currently amended). An isolated polypeptide which is
(1) a polypeptide consisting of an amino acid sequence which is (a) identical to residues 234-1791 of SEQ ID NO:2, or (b) a fragment, at least 5 ~~6~~ amino acids in

length, of mature PAPP-A2 (residues 234-1791 of SEQ ID NO:2),

where said fragment

i) has a proteolytic activity specific at least for Insulin Like Growth Factor Binding Protein 5 (IGFBP-5); and/or

ii) is recognized by an antibody, or a binding fragment thereof, which recognizes mature PAPP-A2 ~~a polypeptide having the amino acid sequence as shown in SEQ ID NO:2; and/or~~

iii) competes with mature PAPP-A2 ~~a polypeptide having the amino acid sequence as shown in SEQ ID NO:2~~ for binding to a cell surface receptor with an affinity for said mature PAPP-A2; ~~polypeptide~~

and where said fragment comprises

at least one of the following regions of SEQ ID NO:2:

Cys-403 to Cys-499

Cys-828 to Cys-881

Cys-1048 to Cys-1115

Cys-1390 to Cys-1396

Cys-1459 to Cys-1464

Cys-1521 to Cys-1525

Cys-1590 to Cys-1595

Cys-1646 to Cys-1653

Cys-1729 to Cys-1733

or

(2) a polypeptide which consists of a fusion of the polypeptide of (1) with an immunogenic carrier protein, or with a tag which may be used to facilitate the detection or purification of the fusion, ~~with the proviso that said fusion of (2) is not a pregnancy-associated plasma protein.~~

91 (currently amended). The polypeptide of claim 90 which is a fragment according to (1)(b).

92 (currently amended). The polypeptide of claim 91 which is a fragment, at least 17 amino acids in length, of mature PAPP-A2 (residues 234-1791 of SEQ ID NO:2).

93 (currently amended). The polypeptide of claim 91 which comprises at least 1169 consecutive amino acids 75% of the polypeptide (mature PAPP-A2) consisting of residues 234-1791 of the polypeptide of SEQ ID NO:2 ~~mature PAPP-A2~~.

94 (cancelled).

95 (currently amended). The polypeptide of claim 91 which comprises the elongated zinc binding consensus sequence (amino acids 733 to 743 of SEQ ID NO:2), LNR 1-3, SCR 1-5, (amino acids 586 to 612 of SEQ ID NO:2), LNR 2 (amino acids 619 to 644 of SEQ ID NO:2), LNR 3 (amino acids 1733 to 1758 of SEQ ID NO:2), SCR1 (amino acids 1396 to 1459 of SEQ ID NO:2), SCR2 (amino acids 1464 to 1521 of SEQ ID NO:2), SCR3 (amino acids 1525 to 1590 of SEQ ID NO:2), SCR4 (amino acids 1595 to 1646 of SEQ ID NO:2), SCR5 (amino acids 1653 to 1729 of SEQ ID NO:2), and all cysteine residues of mature PAPP-A2.

96 (currently amended). The polypeptide of claim 90 where the fragment is at least 50 ~~AA~~ amino acids in length.

97 (currently amended). An isolated polypeptide comprising (I) amino acids 1-22 of SEQ ID NO:2, and
(II) a sequence selected from the group consisting of

(a) a sequence consisting of residues 234-1791 of SEQ ID NO:2, and

(b) a sequence which is at least 95% identical to (a) above,

where said polypeptide, or a cleavable fragment corresponding to sequence (II) of said polypeptide,

i) has a proteolytic activity against Insulin Like Growth Factor Binding Protein 5 (IGFBP-5); and/or

ii) is recognised by an antibody, or a binding fragment

thereof, which recognizes a mature pregnancy associated plasma protein A-2 (mature PAPP-A2) polypeptide consisting of the amino acid sequence shown as amino acids 234-1791 of SEQ ID NO:2.

98 (currently amended). An isolated polypeptide comprising (I) amino acids 23-233 of SEQ ID NO:2, and

(II) a sequence selected from the group consisting of

(a) a sequence consisting of residues 234-1791 of SEQ ID NO:2, and

(b) a sequence which is at least 95% identical to (a) above,

where said polypeptide, or a cleavable fragment corresponding to sequence (II) of said polypeptide,

i) has a proteolytic activity against Insulin Like Growth Factor Binding Protein 5 (IGFBP-5); and/or

ii) is recognised by an antibody, or a binding fragment thereof, which recognizes a mature pregnancy associated plasma protein A-2 (mature PAPP-A2) polypeptide consisting of the amino acid sequence shown as amino acids 234-1791 of SEQ ID NO:2.

99 (currently amended). An isolated polypeptide comprising (I) amino acids 1-233 of SEQ ID NO:2 and

(II) a sequence selected from the group consisting of

(a) a sequence consisting of residues 234-1791 of SEQ ID NO:2, and

(b) a sequence which is at least 95% identical to (a) above

where said polypeptide, or a cleavable fragment corresponding to sequence (II) of said polypeptide,

i) has a proteolytic activity against Insulin Like Growth Factor Binding Protein 5 (IGFBP-5); and/or

ii) is recognised by an antibody, or a binding fragment thereof, which recognizes a mature pregnancy associated plasma protein A-2 (mature PAPP-A2) polypeptide consisting of the amino acid sequence shown as amino acids 234-1791 of SEQ ID NO:2.

100 (currently amended). The polypeptide of claim 91 which is a ~~processing variant fragment~~ of mature PAPP-A2 which results from intracellular proteolysis.

101 (new). The polypeptide of claim 99 which consists of the amino acid sequence of SEQ ID NO:2.

102 (new). An isolated polypeptide which

- (a) consists of residues 234-1791 of SEQ ID NO:2; or
- (b) is at least 99% identical to a polypeptide of (a), but differs therefrom by one or more insertions and/or deletions and/or substitutions.

103 (new). The polypeptide of claim 102 which has a proteolytic activity against Insulin Like Growth Factor Binding Protein 5 (IGFBP-5).

104 (new). The polypeptide of claim 12, whose amino acid sequence differs from that of a polypeptide according to (a), if at all, solely by one or more amino acid substitutions.

105 (new). The polypeptide of claim 12 where said insertions or deletions, if any, are of 1 to 5 amino acid residues.

106 (new). The polypeptide of claim 12 where said insertions or deletions, if any, are of consecutive N-terminal or C-terminal amino acids.

107 (new). The polypeptide of claim 105 where said insertions or deletions, if any, are of consecutive N-terminal or C-terminal amino acids.

108 (new). The polypeptide of claim 12 which is a fragment of mature PAPP-A2 (234-1791 of SEQ ID NO:2).

109 (new). An isolated polypeptide which (a) consists of

USSN - 09/983,025

residues 234-1791 of SEQ ID NO:2, or

(b) differs from the polypeptide of (a) solely by

(b1) deletion of 1-10 residues from, or addition of 1-10 residues to, the amino terminal, and/or

(b2) deletion of 1-10 residues from, or addition of 1-10 residues to, the carboxy terminal.